## CLAIMS

1. A method of increasing the specificity of a PCR-based bacterial assay, the method comprising

aligning a chosen non-*E. coli* bacterial target nucleic acid sequence with a homologous *E. coli* nucleic acid sequence;

selecting a PCR primer sequence such that it comprises a sequence perfectly complementary in its three 3'-terminal nucleotides to the chosen non-*E. coli* bacterial target nucleic acid sequence, and one or more mismatches, in its three 3'-terminal nucleotides, to said homologous *E. coli* nucleic acid sequence; and

performing PCR using said PCR primer sequence in a PCR-based bacterial assay.

2. The method of claim 1, wherein said primer sequence comprises two or more mismatches in its three 3'-terminal nucleotides, to said corresponding *E. coli* nucleic acid sequence.

3. The method of claim 1, wherein said primer sequence comprises three mismatches in its three 3'-terminal nucleotides, to said corresponding *E. coli* nucleic acid sequence.

4. A composition comprising an oligonucleotide primer that hybridizes under standard conditions to a nucleic acid sequence comprised by a *Mycoplasma* 16S rRNA gene, wherein the 3'-terminal two nucleotides of the primer are selected so as not to base pair with template if the oligonucleotide primer cross-hybridizes with an *E. coli* 16S rRNA gene template.

5. The composition of claim 4 wherein said primer hybridizes to a 16S rRNA gene sequence from at least two species of *Mycoplasma*.

6. The composition of claim 4 wherein said primer hybridizes to a 16S rRNA gene sequence from at least three species of *Mycoplasma*.

7. The composition of claim 4 wherein said primer hybridizes to a 16S rRNA gene sequence from at least four species of *Mycoplasma*.

8. The composition of claim 4 wherein said primer hybridizes to a 16S rRNA gene sequence from at least five species of *Mycoplasma*.

9. The composition of claim 4 wherein said primer hybridizes to a 16S rRNA gene sequence from at least six species of *Mycoplasma*.

10. The composition of claim 4 wherein said primer hybridizes to a 16S rRNA gene sequence from at least seven species of *Mycoplasma*.

11. The composition of claim 4 wherein said primer hybridizes to a 16S rRNA gene sequence from at least eight species of *Mycoplasma*.

12. The composition of claim 4 that comprises at least two oligonucleotide primers that hybridize to opposite strands of a nucleic acid sequence comprised by a *Mycoplasma* 16S rRNA gene, wherein the 3'-terminal two nucleotides of at least one of the primers are selected so as not to base pair with template if the at least one oligonucleotide primer cross-hybridizes with an *E. coli* 16S rRNA gene template.

13. The composition of claim 4, further comprising an internal amplification control template.

14. The composition of claim 13 wherein said internal amplification control template has the sequence of SEQ ID NO: 5 or the complement thereof.

15. The composition of claim 4 wherein said oligonucleotide primer has a sequence selected from the group consisting of (SEQ ID Nos 1-4).

16. A kit comprising a composition of claim 4.

17. The kit of claim 16, further comprising an internal amplification control template nucleic acid.

18. The kit of claim 17 wherein said internal amplification control template has the sequence of SEQ ID NO 5 or the complement thereof.

19. The kit of claim 16 further comprising a template-dependent nucleic acid extending enzyme.

20. The kit of claim 16 further comprising a uracil DNA glycosylase enzyme.

21. An isolated nucleic acid consisting of the sequence of any one of SEQ ID NOs 1-4 or the respective complement thereof.

22. An isolated nucleic acid comprising the sequence of SEQ ID NO: (5) or the complement thereof.

23. A kit comprising an isolated nucleic acid of claim 21.

24. The kit of claim 23 further comprising an internal amplification control nucleic acid template.

25. The kit of claim 24 wherein said internal amplification control nucleic acid template comprises the sequence of SEQ ID NO (5) or the complement thereof.

26. The kit of claim 23 further comprising a template-dependent nucleic acid extending enzyme.

27. The kit of claim 23 further comprising a uracil DNA glycosylase enzyme.

28. A method of detecting the presence of a *Mycoplasma* species in a sample, the method comprising:

forming a reaction mixture comprising the sample and a set of oligonucleotide primers that hybridize under standard conditions to a nucleic acid sequence comprised by a *Mycoplasma* 16S rRNA gene, wherein the 3'-terminal nucleotide of one or more of said set of primers is selected so as not to base pair with an *E. coli* 16S rRNA gene template if the oligonucleotide primer cross-hybridizes with said *E. coli* 16S rRNA gene template;

extending said primers; and

detecting extension products of said primers, wherein the presence of an extension product is indicative of the presence of a *Mycoplasma* species in said sample.

29. The method of claim 28 wherein the 3'-terminal two nucleotides of one or more of said set of primers are selected so as not to base pair with an *E. coli* 16S rRNA gene template if said one or more of said set of oligonucleotide primers cross-hybridizes with said *E. coli* 16S rRNA gene template.

30. The method of claim 28 wherein the step of extending said primers comprises polymerase chain reaction (PCR) amplification.

31. The method of claim 30 wherein the reaction mixture further comprises an internal amplification control template, wherein the product of amplification of the internal amplification control template is detectably different in size or sequence than the product of amplification of *Mycoplasma* 16S rRNA species amplified in said sample.

32. The method of claim 31 wherein said internal amplification control comprises a nucleic acid template comprising 5' and 3' regions that hybridize with corresponding regions of a 16S rRNA gene sequence from one or more *Mycoplasma* species under standard conditions, flanking a central region of non-16S rRNA gene sequence, wherein said 5' and 3' regions hybridize to oligonucleotide primers in said primer set.

33. The method of claim 31 wherein said internal amplification control template comprises the sequence of SEQ ID NO. 5.

34. The method of claim 28 further comprising the step, before the step of extending said primers, of contacting said reaction mixture with a uracil DNA glycosylase enzyme.

35. The method of claim 28 wherein said reaction mixture comprises dUTP.

36. The method of claim 28 wherein the step of extending said primers is performed in the presence of dUTP, and wherein said extending results in the incorporation of dUTP into an extension product.

37. The method of claim 28 wherein the step of detecting extension products comprises gel electrophoresis.

38. The method of claim 28 wherein the set of oligonucleotide primers comprises a primer selected from the group consisting of: (SEQ ID Nos: 1-4).

39. The method of claim 28 wherein the set of oligonucleotide primers comprises SEQ ID Nos: 1 & 2.

40. The method of claim 28 wherein the set of oligonucleotide primers consists of SEQ ID Nos: 1-4.

41. The method of claim 28 wherein the set of oligonucleotide primers detects the presence of *Mycoplasma* species including *Acholeplasma laidlawii*, *Mycoplasma arginini*, *M. fermentans*, *M. hominis*, *M. hyorhinis*, *M. orale*, *M. salivarium, and M. pirum.*

42. A method for detecting the presence of a *Mycoplasma* species in a sample, the method comprising:

contacting the sample with a set of oligonucleotide primers that hybridize to a 16S rRNA gene sequence in one or more *Mycoplasma* species, the set of oligonucleotide primers comprising SEQ ID NO: 2;

extending at least one of said primers; and

detecting an extension product, wherein the presence of an extension product indicates the presence of a *Mycoplasma* species in said sample.

43. The method of claim 42, wherein said set of oligonucleotide primers further comprises a primer selected from the group consisting of SEQ ID NOs 1, 3 and 4.

44. The method of claim 42, wherein said set of oligonucleotide primers further comprises oligonucleotide primers of SEQ ID NOs 1 & 3.

45. The method of claim 42, wherein said set of oligonucleotide primers further comprises oligonucleotide primers of SEQ ID NOs 1 & 4.

46. The method of claim 42, wherein said set of oligonucleotide primers further comprises oligonucleotide primers of SEQ ID NOs 1, 3 & 4.

47. The method of claim 42 wherein for at least one member of the set of oligonucleotide primers, the 3'-terminal nucleotide is selected such that it does not base pair with an *E. coli* 16S rRNA gene template if said member cross-hybridizes with an *E. coli* 16S rRNA gene template.

48. The method of claim 47 wherein for at least one member of the set of oligonucleotide primers, the 3'-terminal two nucleotides are selected such that they do not base pair with an *E. coli* 16S rRNA gene template if said member cross-hybridizes with an *E. coli* 16S rRNA gene template.

49. The method of claim 42 wherein the step of extending said primers comprises polymerase chain reaction (PCR) amplification.

50. The method of claim 42 further comprising the step, either before, or concurrent with the step of contacting the sample with a set of oligonucleotides, of adding an internal control template to said sample.

51. The method of claim 50 wherein the extension product of said internal control template is detectably different in size or sequence from any extension product resulting from extension of said set of primers on a *Mycoplasma* 16S rRNA gene nucleic acid template.

52. The method of claim 42 wherein said internal amplification control template comprises the sequence of SEQ ID NO. 5.

53. The method of claim 42 further comprising the step, before the step of extending said primers, of contacting said reaction mixture with a uracil DNA glycosylase enzyme.

54. The method of claim 42 wherein said reaction mixture comprises dUTP.

55. The method of claim 42 wherein the step of extending said primers is performed in the presence of dUTP, and wherein said extending results in the incorporation of dUTP into an extension product.

56. The method of claim 42 wherein the step of detecting extension products comprises gel electrophoresis.

57. The method of claim 42 wherein the set of oligonucleotide primers detects the presence of *Mycoplasma* species including *Acholeplasma laidlawii*, *Mycoplasma arginini*, *M. fermentans*, *M. hominis*, *M. hyorhinis*, *M. orale*, *M. salivarium,* and *M. pirum*.

58. A method for detecting the presence of a *Mycoplasma* species in a sample, the method comprising:

contacting the sample with a set of oligonucleotide primers that hybridize to a 16S rRNA gene sequence in one or more *Mycoplasma* species, the set of oligonucleotide primers comprising SEQ ID NO. 1.

extending at least one of said primers;

detecting an extension product, wherein the presence of an extension product indicates the presence of a *Mycoplasma* species in said sample.

59. The method of claim 58, wherein said set of oligonucleotide primers further comprises a primer selected from the group consisting of SEQ ID NOs 2, 3, & 4.

60. The method of claim 58, wherein said set of oligonucleotide primers further comprises oligonucleotide primers of SEQ ID NOs 3 & 2.

61. The method of claim 58, wherein said set of oligonucleotide primers further comprises oligonucleotide primers of SEQ ID NOs 4 & 2.

62. The method of claim 58, wherein said set of oligonucleotide primers further comprises oligonucleotide primers of SEQ ID Nos 2, 3 & 4.

63. The method of claim 58 wherein for at least one member of the set of oligonucleotide primers, the 3'-terminal nucleotide is selected such that it does not base pair with an *E. coli* 16S rRNA gene template if said member cross-hybridizes with an *E. coli* 16S rRNA gene template.

64. The method of claim 63 wherein for at least one member of the set of oligonucleotide primers, the 3'-terminal two nucleotides are selected such that they do not base pair with an *E. coli* 16S rRNA gene template if said member cross-hybridizes with an *E. coli* 16S rRNA gene template.

65. The method of claim 58 wherein the step of extending said primers comprises polymerase chain reaction (PCR) amplification.

66. The method of claim 58 further comprising the step, either before, or concurrent with the step of contacting the sample with a set of oligonucleotides, of adding an internal control template to said sample.

67. The method of claim 66 wherein the extension product of said internal control template is detectably different in size or sequence from any extension product resulting from extension of said set of primers on a *Mycoplasma* 16S rRNA gene nucleic acid template.

68. The method of claim 58 wherein said internal amplification control template comprises the sequence of SEQ ID NO. 5.

69. The method of claim 58 further comprising the step, before the step of extending said primers, of contacting said reaction mixture with a uracil DNA glycosylase enzyme.

70. The method of claim 58 wherein said reaction mixture comprises dUTP.

71. The method of claim 58 wherein the step of extending said primers is performed in the presence of dUTP, and wherein said extending results in the incorporation of dUTP into an extension product.

72. The method of claim 58 wherein the step of detecting extension products comprises gel electrophoresis.

73. The method of claim 58 wherein the set of oligonucleotide primers detects the presence of *Mycoplasma* species including *Acholeplasma laidlawii*, *Mycoplasma arginini*, *M. fermentans*, *M. hominis*, *M. hyorhinis*, *M. orale*, *M. salivarium,* and *M. pirum*.

74. A method for detecting the presence of a *Mycoplasma* species in a sample, the method comprising:

contacting the sample with a set of oligonucleotide primers that hybridize to a 16S rRNA gene sequence in one or more *Mycoplasma* species, the set of oligonucleotide primers comprising SEQ ID NO. 3;

extending at least one of said primers; and

detecting an extension product, wherein the presence of an extension product indicates the presence of a *Mycoplasma* species in said sample.

75. The method of claim 74, wherein said set of oligonucleotide primers further comprises a primer selected from the group consisting of SEQ ID NOs 1, 2 & 4.

76. The method of claim 74, wherein said set of oligonucleotide primers further comprises oligonucleotide primers of SEQ ID NOs 1 & 2.

77. The method of claim 74, wherein said set of oligonucleotide primers further comprises oligonucleotide primers of SEQ ID NOs 4 & 2.

78. The method of claim 74, wherein said set of oligonucleotide primers further comprises oligonucleotide primers of SEQ ID NOs 1, 2 & 4.

79. The method of claim 74 wherein for at least one member of the set of oligonucleotide primers, the 3'-terminal nucleotide is selected such that it does not base pair with an *E.*

*coli* 16S rRNA gene template if said member cross-hybridizes with an *E. coli* 16S rRNA gene template.

80. The method of claim 79 wherein for at least one member of the set of oligonucleotide primers, the 3'-terminal two nucleotides are selected such that they do not base pair with an *E. coli* 16S rRNA gene template if said member cross-hybridizes with an *E. coli* 16S rRNA gene template.

81. The method of claim 74 wherein the step of extending said primers comprises polymerase chain reaction (PCR) amplification.

82. The method of claim 74 further comprising the step, either before, or concurrent with the step of contacting the sample with a set of oligonucleotides, of adding an internal control template to said sample.

83. The method of claim 82 wherein the extension product of said internal control template is detectably different in size or sequence from any extension product resulting from extension of said set of primers on a *Mycoplasma* 16S rRNA gene nucleic acid template.

84. The method of claim 74 wherein said internal amplification control template comprises the sequence of SEQ ID NO. 5.

85. The method of claim 74 further comprising the step, before the step of extending said primers, of contacting said reaction mixture with a uracil DNA glycosylase enzyme.

86. The method of claim 74 wherein said reaction mixture comprises dUTP.

87. The method of claim 74 wherein the step of extending said primers is performed in the presence of dUTP, and wherein said extending results in the incorporation of dUTP into an extension product.

88. The method of claim 74 wherein the step of detecting extension products comprises gel electrophoresis.

89. The method of claim 74 wherein the set of oligonucleotide primers detects the presence of *Mycoplasma* species including *Acholeplasma laidlawii*, *Mycoplasma arginini*, *M. fermentans*, *M. hominis*, *M. hyorhinis*, *M. orale*, *M. salivarium,* and *M. pirum*.

90. A method for detecting the presence of a *Mycoplasma* species in a sample, the method comprising:

contacting the sample with a set of oligonucleotide primers that hybridize to a 16S rRNA gene sequence in one or more *Mycoplasma* species, the set of oligonucleotide primers comprising SEQ ID NO. 4;

extending at least one of said primers; and

detecting an extension product, wherein the presence of an extension product indicates the presence of a *Mycoplasma* species in said sample.

91. The method of claim 90 further comprising the step, either before, or concurrent with the step of contacting the sample with a set of oligonucleotides, of adding an internal control template to said sample.

92. The method of claim 91 wherein the extension product of said internal control template is detectably different in size or sequence from any extension product resulting from extension of said set of primers on a *Mycoplasma* 16S rRNA gene nucleic acid template.

93. The method of claim 90, wherein said set of oligonucleotide primers further comprises a primer selected from the group consisting of SEQ ID NOs 1, 2 & 3.

94. The method of claim 90, wherein said set of oligonucleotide primers further comprises oligonucleotide primers of SEQ ID NOs 1 & 2.

95. The method of claim 90, wherein said set of oligonucleotide primers further comprises oligonucleotide primers of SEQ ID NOs 3 & 2.

96. The method of claim 90, wherein said set of oligonucleotide primers further comprises oligonucleotide primers of SEQ ID Nos 1, 2 & 3.

97. The method of claim 90 wherein for at least one member of the set of oligonucleotide primers, the 3'-terminal nucleotide is selected such that it does not base pair with an *E. coli* 16S rRNA gene template if said member cross-hybridizes with an *E. coli* 16S rRNA gene template.

98. The method of claim 97 wherein for at least one member of the set of oligonucleotide primers, the 3'-terminal two nucleotides are selected such that they do not base pair with an *E. coli* 16S rRNA gene template if said member cross-hybridizes with an *E. coli* 16S rRNA gene template.

99. The method of claim 90 wherein the step of extending said primers comprises polymerase chain reaction (PCR) amplification.

100. The method of claim 90 wherein said internal amplification control template comprises the sequence of SEQ ID NO. 5.

101. The method of claim 90 further comprising the step, before the step of extending said primers, of contacting said reaction mixture with a uracil DNA glycosylase enzyme.

102. The method of claim 90 wherein said reaction mixture comprises dUTP.

103. The method of claim 90 wherein the step of extending said primers is performed in the presence of dUTP, and wherein said extending results in the incorporation of dUTP into an extension product.

104. The method of claim 90 wherein the step of detecting extension products comprises gel electrophoresis.

105. The method of claim 90 wherein the set of oligonucleotide primers detects the presence of *Mycoplasma* species including *Acholeplasma laidlawii*, *Mycoplasma arginini*, *M. fermentans*, *M. hominis*, *M. hyorhinis*, *M. orale*, *M. pirum*, and *M. salivarium*.